New Standards in Collagen Quantification: The Value of Second Harmonic Generation

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Genesis Imaging Services
PharmaNest, Princeton, NJ
Genesis Imaging Services

Current Capabilities

Two Optical Methods fused in one instrument

5 micron
FFPE
Non-Stained
Or
Frozen

Imaging
(Genesis200®)
(Laennec®)

SHG
Concurrent
Imaging

2PE
Concurrent
multiparametric
quantification

Molecular Biology or Other markers

Histolindex, Singapore
Rodent Liver / NASH
Green = Collagen
Red = NADPH
Zoomed in
Rodent Liver / NASH
Green = Collagen
ONLY
Zoomed in
IPF: Bleomycin Induced Lung Fibrosis Treated with or without Nintedanib or Pirfenidone
IPF: Bleomycin Induced Lung Fibrosis Treated with or without Nintedanib or Pirfenidone

Fibrosis Content and Structure

<table>
<thead>
<tr>
<th>Collagen Content (CAR)</th>
<th>Ctrl</th>
<th>BLM</th>
<th>NTN</th>
<th>PFD</th>
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<th>Collagen Structure (CRI)</th>
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<th>3.0</th>
<th>3.5</th>
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Fibrosis Regional Quantification

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<th>Lesional %</th>
<th>Parenchymal %</th>
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<td>4</td>
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Kidney: UUO Fibrosis in Rat
Treated with or without Pirfenidone

Control  UUO  UUO + PFD

Cortex

Medulla
Kidney: UUO Fibrosis in Rat

Rodent Model Dynamic Range Selection

Collagen Structure

Collagen Content
Kidney: UUO Fibrosis in Rat
Treated with or without Pirfenidone

2D Fibrosis (Total)

Collagen Content (CAR)

Collagen Structure (CRI)

Sham
UUO
UUO+PFD

Sirius Red
H&E

Sirius Red

Hydroxyproline

Ctrl
PFD
UUO

Kidney: UUO Fibrosis in Rat
Treated with or without Pirfenidone
Liver: NASH (Modified ALIOS diet-feed mice)

Label Free, Automated, Concurrent Fibrosis and Steatosis Quantification

2D Fibrosis-Fat Chart

Collagen Content

Fat Content

Area/Droplet (pixel)

NASH

Drug A

Drug B

CHOW

Princeton, NJ
November 13-14, 2017
Liver: NASH (Modified ALIOS diet-feed mice)

Concordance with Standard Methods (50% less N)
Liver: NASH (Modified ALIOS diet-feed mice)

Concordance with Standard Methods (50% less N)

Data from conventional method is provided by Intercept.
Truly Quantitative Fibrosis Parameters (qFPs)

Septal Bridges Classification

Septal Bridge Characteristics

<table>
<thead>
<tr>
<th>Width</th>
<th>Branches</th>
<th>Segment Length</th>
<th>Collagen Reticulation Index</th>
<th>Collagen Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>at 1/3 &amp; 2/3 intersection</td>
<td></td>
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CCl4 (4wk)  
CCl4 (6wk)  
CCl4+PFD (6wk)

Septal Width

Septal Collagen Area%
Truly Quantitative Fibrosis Parameters (qFPs)

Fibrosis Morphometric Phenotypic <Models

- Area
- Perimeter
- Compactness
- Length
- Breadth
- Convex Hull Perimeter
- Convex Hull Area
- Elongation
- Roughness
- Major Axis Length
- Minor Axis Length
- Orientation
- Axis Ratio
- Tortuosity

16 different qFPs

The qFP fibrosis score provides a continuous metric for the assessment of fibrosis, that is independent of staining artifacts and reader variability.

DUAL PHOTON MICROSCOPY BASED QUANTITATION OF FIBROSIS-RELATED PARAMETERS (Q-FP) TO MODEL DISEASE PROGRESSION IN STEATOHEPATITIS. Yan Wang MD PhD, Hepatology. doi: 10.1002/hep.29090
Fibrosis Phenotypic Morphometric Modeling
Based on Collagen Morphometrics features

Morphometric Phenotype Chart

Each column represents and quantifies a stage:

- F progression
- Time course
- Drug A vs Drug B vs Chow
- Animal Model 1 vs Animal Model 2
- Animal vs human
- AHS vs NASH
- Obese vs Diabetes vs Normal
New standards in Fibrosis Quantification

The Value of Second Harmonic Generation

- Requires Biopsy, compatible with existing pathology workflows, eliminates staining and reader variability. Collagen I and III optical biomarker.

- Generates a 100% specific BigTIFF image for Collagen I and III (SHG) allowing fully quantitative and morphometric fibrosis quantification (content and structure)

- Validated for most pre-clinical models (Liver, NASH, Kidney, IPF)
- Correlate with standard methods (with at 50% N)
- Basic Quantification at “Pathology +20% - 30%” price premium
- 2 weeks turnaround (N=40)

- Growing panels of robust and validated Drug Discovery Tools
- Fully Translational
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PharmaNest, Princeton, NJ
March 9, 2016

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